

EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION
ORGANISATION EUROPEENNE ET MEDITERRANEENNE POUR LA PROTECTION DES PLANTES
Summary sheet of validation data for a diagnostic test

The EPPO Standard PM 7/98 *Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity* describes how validation should be conducted. It also includes definitions of performance criteria.

Laboratory contact details	ILVO Institute for Agricultural and Fisheries Research Burg. Van Gansberghelaan 96, 9820 Merelbeke - Melle, Belgium
Short description of the test	Identification of <i>Frankliniella occidentalis</i> using COI barcoding
Date, reference of the validation report	2024-06-03 -
Validation process according to EPPO Standard PM7/98?	no
Is the lab accredited for this test?	no
Was the validated data generated in the framework of a project?	no
Description of the test	
Organism(s)	<i>Frankliniella occidentalis</i> (FRANOC)
Detection / identification	identification
Matrix(ces) tested	Specimen
Method(s)	Molecular Sanger seq
Method: Molecular Sanger seq	
Reference of the test description	
As or adapted from an EPPO diagnostic protocol	yes
Other information	
Other details on the test	PM 7/129 COI loci
Are the performance characteristics included in the EPPO diagnostic protocol?	no
Performance Criteria :	
Organism 1.:	<i>Frankliniella occidentalis</i>(FRANOC)
Analytical sensitivity	
What is the smallest amount of target that can be detected reliably?	PCR reaction not evaluated
Diagnostic sensitivity	
Proportion of infected/infested samples	PCR reaction not evaluated

tested positive compared to results from the standard test, see appendix 2 of PM 7/98	
Analytical specificity - inclusivity	
Number of strains/populations of target organisms tested	Phylogenetic analysis: 31 <i>F. occidentalis</i> sequences including 21 from EPPO-Q-bank and 10 from BOLD
Specificity value	100%
Analytical specificity - exclusivity	
Number of non-target organisms tested	Phylogenetic analysis performed on 89 sequences from EPPO-Q-bank covering the following species: <i>Frankliniella insularis</i> (Fins) <i>Frankliniella tenuicornis</i> (Ften) <i>Scirtothrips aurantii</i> (Saur) <i>Scirtothrips citri</i> (Scit) <i>Scirtothrips dorsalis</i> (Sdor) <i>Scirtothrips perseae</i> (Sper) <i>Thrips major</i> (Tmaj) <i>Thrips nigropilosus</i> (Tnig) <i>Thrips palmi</i> (Tpal) <i>Thrips tabaci</i> (Ttab) and 170 sequences from BOLD <i>Frankliniella tritici</i> (Ftri) 10 <i>Frankliniella panamensis</i> (Fpan) 10 <i>Frankliniella citripes</i> (Fcit) 1 <i>Frankliniella schultzei</i> (Fsch) 10 <i>Frankliniella gossypiana</i> (Fgos) 10 <i>Frankliniella insularis</i> (Fins) 10 <i>Frankliniella invasor</i> (Finv) 10 <i>Frankliniella intonsa</i> (Fint) 8 <i>Frankliniella gardeniae</i> (Fgar) 10 <i>Frankliniella fusca</i> (Ffus) 10 <i>Frankliniella andrei</i> (Fand) 9 <i>Frankliniella brunnea</i> (Fbru) 4 <i>Frankliniella minuta</i> (Fmin) 8 <i>Frankliniella unicolor</i> (Funi) 3 <i>Frankliniella tenuicornis</i> (Ften) 10 <i>Frankliniella borinquen</i> (Fbor) 7 <i>Frankliniella cephalica</i> (Fecp) 10 <i>Frankliniella bruneri</i> (Fbru) 2 <i>Frankliniella bispinosa</i> (Fbis) 10 <i>Frankliniella auripes</i> (Faur) 2 <i>Frankliniella rostrata</i> (Fros) 1 <i>Frankliniella parvula</i> (Fpar) 1 <i>Frankliniella fallaciosa</i> (Ffal) 1 <i>Frankliniella xanthaner</i> (Fxa) 3 <i>Frankliniella gemina</i> (Fgem) 1 <i>Frankliniella kelliiae</i> (Fkel) 4 <i>Frankliniella australis</i> (Faus) 1 <i>Frankliniella longipennis</i> (Flon) 1 <i>Frankliniella regia</i> (Freg) 2 <i>Frankliniella aztecus</i> (Fazt) 1
Specificity value	100% There is one COI sequence of <i>F. borinquen</i> (Fbor, red dot) that clusters with <i>F. occidentalis</i> . However, as 1) <i>F. borinquen</i> is very similar to <i>F. occidentalis</i> , 2) the sequence for this <i>F. borinquen</i> specimen was derived from NCBI, and 3) as other <i>F. borinquen</i> sequences cluster together at another location in the tree (other red dots), this is probably an incorrectly identified specimen. The sequence was thus not considered for the calculation of the inclusivity
Test performance study	
Test performance study?	no
Other information	
Any other information considered useful	For further Wannes Dermauw : wannes.dermauw@ilvo.vlaanderen.be Jochem Bonte: jochem.bonte@ilvo.vlaanderen.be The wet lab part of the test (PCR) was not evaluated. --phylogenetic analysis details-- Alignment of 290 sequences using MAFFT with auto settings: % mafft --reorder --auto input IQ-tree details: AUTO

detection model, 2000 UF bootstraps, 2000 SH-aLRT IQtree command: "iqtree -s QBANK_BOLD_merged_Fo_MAFFT.fas -m TEST -bb 2000 -alrt 2000" layout phylogenetic tree: consensus tree of IQtree output was loaded in MEGA X; tree was midpoint rooted; green and red dots were added to Focc and Fbor, respectively; only bootstrap value > 95 are shown Species included in the tree: QBANK (all sequences with a "010#" or "BTQ###" suffix, and "EURL_1600162_Tnig" and "QBOL49_Ttab"; 110 sequences in total) Frankliniella insularis (Fins) Frankliniella occidentalis (Focc) Frankliniella tenuicornis (Ften) Scirtothrips aurantii (Saur) Scirtothrips citri (Scit) Scirtothrips dorsalis (Sdor) Scirtothrips perseae (Sper) Thrips major (Tmaj) Thrips nigropilosus (Tnig) Thrips palmi (Tpal) Thrips tabaci (Ttab)→ BOLD (all other sequences; from each Frankliniella specimen determined at the species level in BOLD, a maximum of 10 (randomly selected) COI sequences was included.; 180 sequences in total; number of sequences for each species is shown) Frankliniella tritici (Ftri) 10 Frankliniella panamensis (Fpan) 10 Frankliniella citripes (Fcit) 1 Frankliniella schultzei (Fsch) 10 Frankliniella gossypiana (Fgos) 10 Frankliniella insularis (Fins) 10 Frankliniella invador (Finv) 10 Frankliniella intonsa (Fint) 8 Frankliniella gardeniae (Fgar) 10 Frankliniella fusca (Ffus) 10 Frankliniella andrei (Fand) 9 Frankliniella brunnea (Fbru) 4 Frankliniella minuta (Fmin) 8 Frankliniella unicolor (Funi) 3 Frankliniella tenuicornis (Ften) 10 Frankliniella borinquen (Fbor) 7 Frankliniella cephalica (Fecp) 10 Frankliniella bruneri (Fbru) 2 Frankliniella bispinosa (Fbis) 10 Frankliniella auripes (Faur) 2 Frankliniella rostrata (Fros) 1 Frankliniella parvula (Fpar) 1 Frankliniella fallaciosa (Ffal) 1 Frankliniella xanthaner (Fxa) 3 Frankliniella gemina (Fgem) 1 Frankliniella kelliiae (Fkel) 4 Frankliniella australis (Faus) 1 Frankliniella longipennis (Flon) 1 Frankliniella regia (Freg) 2 Frankliniella aztecus (Fazt) 1 Frankliniella occidentalis (Focc) 10

The following complementary files are available online:

- [Phylogenetic tree](#)
- [F_occidentalis_barcoding_alignment](#)